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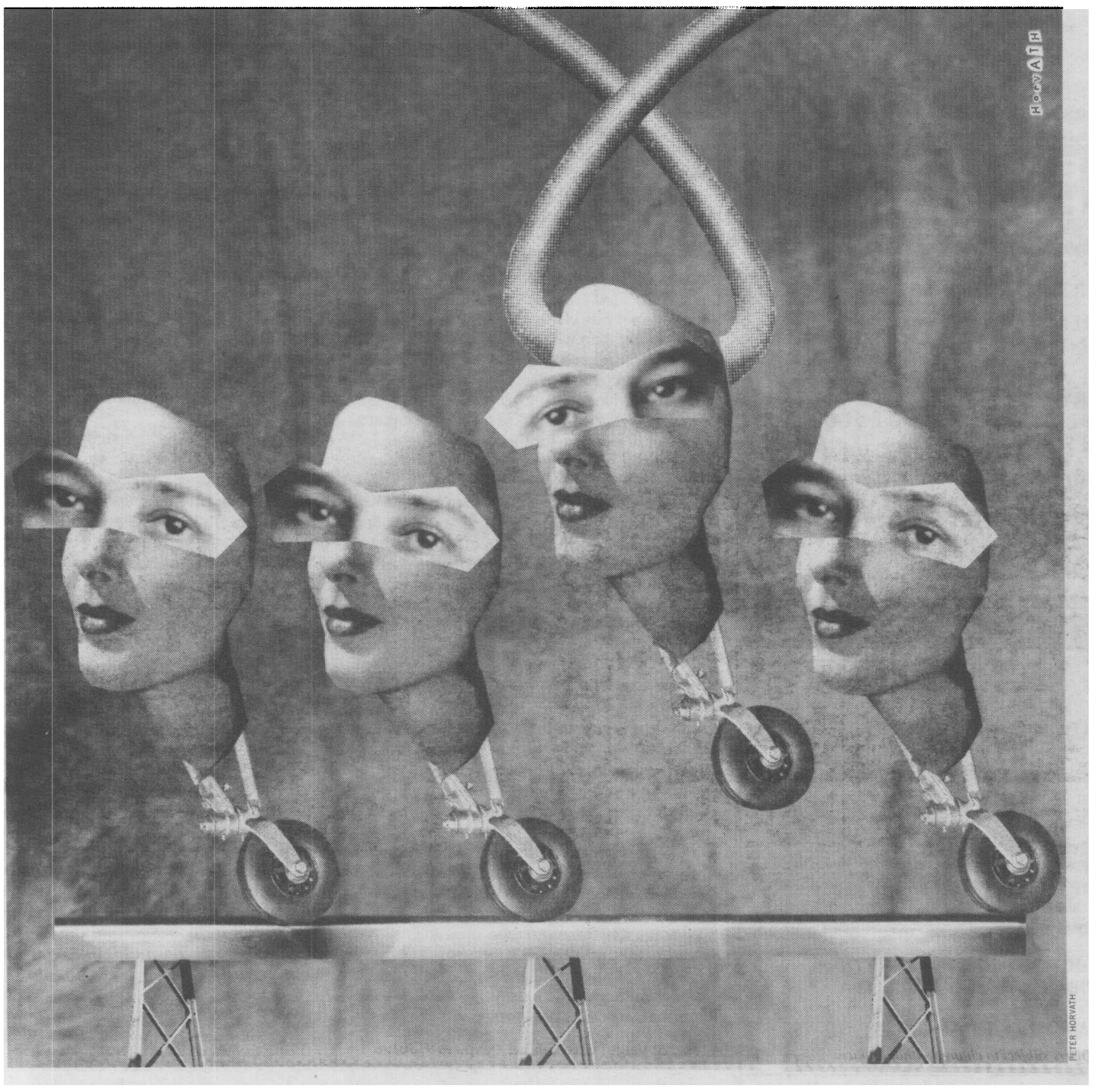
CORRECTING THE DNA CODE



BY MARK SCHOOFS

Michael Blaese is one of the pioneers of gene therapy, the art of curing inherited diseases by mending a patient's DNA. He was the lead investigator of the first federally approved gene-therapy trial—which is also one of the very few actually to help a patient. The field has produced much more hype than healing, and over his 32-year career at the National Institutes of Health, Blaese has seen a lot of bright ideas fizzle.

So what does he think of a method reported just last week, in the prestigious journal *Nature Medicine*, to repair flawed genes? "I think it's so significant that I'm leaving my job at the NIH to go work for the company" that's developing it. "It's what I've been waiting for for the last decade and a half."

Indeed, the new technique might cure some of the most stubborn genetic diseases, such as hemophilia, and lower the odds of contracting others, such as Alzheimer's. It could also be used to improve normal human attributes, such as the ability to metabolize cholesterol, preventing countless cases of heart disease. [*continued on page 44*]

SCHOOFS

CONTINUED FROM PAGE 43

In essence, the discovery is this: Scientists have found a way to correct precise genetic defects. And researchers didn't just do this in a test tube, but in living animals.

THE HUMAN GENETIC code consists of 3 billion molecular letters, strung along DNA's double helix. If a person has hemophilia, for example, just one of those letters is misspelled. Correcting that misspelling requires absolute precision: Changing other letters in the code could cause cancer.

For some years, scientists have known how to make genetic corrections—but the old method, called homologous recombination, works in only about one in 100,000 tries. That's impractical because, to treat a patient, the DNA inside millions of cells would need to be repaired.

No wonder W. French Anderson, one of the grand old men of gene therapy, recently said, "We're years if not decades from being able to do true genetic surgery, where you go in and repair a genetic defect." But he was speaking four months ago, and genetics is moving so fast it sometimes surprises even its practitioners.

In the experiment reported this month, researchers at the University of Minnesota injected rats with special molecules, which floated through the bloodstream until they reached the liver. There, these molecules entered liver cells and changed one letter of the code in the gene for factor IX, a protein necessary for blood to clot.

The rat gene for factor IX is almost identical to the human gene, but rats don't get the mutation that causes hemophilia. Therefore, researchers obviously couldn't correct it, so they did the opposite: They caused it by inducing the misspelling that produces the disease. In this way, they proved that targeted alteration of genes can be accomplished.

How well did it work? The gene was rewritten in a whopping 40 per cent of the target cells—and lead researcher Clifford J. Steer says that in more recent, unpublished experiments, his team has managed to push up to 60 per cent. That is orders of magnitude better than the old rate of one in 100,000—and many times better than what would be needed to cure hemophilia. (Correcting just 5 to 10 per cent of liver cells in hemophiliacs would be enough to make their blood able to clot.)

"This is great news," says George Omburo, director of research for the National Hemophilia Foundation. Currently, many hemophiliacs must inject themselves every week with clotting factors, which can cost more than $100,000 a year. And because the clotting factors are often made from human blood, hemophiliacs have been vulnerable to emerging viruses such as HIV and hepatitis C. Though it may take many years before the new gene-repair technique actually helps patients, Omburo says, "It's a very big breakthrough."

BUT IS IT FOR REAL? The fundamental process was reported in *Science* 18 months ago—and many scientists flat out didn't believe it. At that point it had only been tried in the test tube, correcting the misspelling that causes sickle-cell anemia at rates so much better than homologous recombination that it seemed too good to be true.

At the center of this storm is the scientist who invented the process, Eric Kmiec. But it was his three-year-old son who gave him the idea.

Kmiec knew that if two strands of DNA match, letter for letter, then they will adhere, "sort of like a zipper." But if one of the strands is short, they don't adhere very tightly, and the zipper pulls apart all too easily. One of Kmiec's graduate students was struggling to figure out how to strengthen the adhesion; at the time, no one foresaw that solving this problem would lead to correcting genetic defects.

One day Kmiec was pondering this little puzzle while driving his tractor mower on his farm. "My son was sitting on my lap, and I was blabbing and blabbing about genes and what to do about this problem, and of course he wasn't paying any attention to me. But suddenly he said, probably referring to something in his own world, 'Just put the things together.' "

Kmiec stopped the mower, called his lab colleagues, and suggested that they put DNA and RNA together. It worked: For some reason, adding a bit of RNA makes the DNA zipper adhere securely. Kmiec calls this a "chimeric RNA/DNA molecule." (Scientists use the word *chimeric* to describe chemical grafting.)

But as it turns out, this chimeric molecule does something even more amazing: It can reconcile any discrepancies between itself and the matching strand of DNA, "correcting the typo," as Blaese puts it. It can also add a letter that is missing, or delete one that shouldn't be there. No one knows for sure how this happens, but it appears that the molecule marshals the cell's own DNA repair mechanism.

Just as important, the chimeric molecule doesn't seem to make any changes where it shouldn't. If it has three or more letters that don't match the target stretch of DNA, then it doesn't seem to bind at all, and so the repair process never gets started. In sum, this simple molecule apparently induces perfectly targeted corrections, what Anderson calls "true genetic surgery."

That's what Kmiec published a year and a half ago, and that's what scientists thought was too good to be true. Steer's experiment tips the balance in Kmiec's favor—it repeats his findings, and extends them to live animals. But the jury is still out, partly because several other scientists have failed to achieve the same results.

One such researcher is Michael Strauss, a professor at the Humboldt University in Berlin, Germany. He penned a commentary that accompanied this month's experiment, calling the new work "both very inspiring and very controversial." In other words, it could be a fluke.

But the truth will soon be known, because many researchers will now try the technique. "If it doesn't hold up," chuckles Steer, "I will enroll in the witness protection program and hope my kids remember me well."

STEER IS CONFIDENT: "This is not cold fusion," he says, "this is real." If so, why was he able to replicate Kmiec's findings when others couldn't?

Maybe he picked the right gene. Inside cells, DNA is tightly coiled, and some genes might be folded up in such a way that they can't be reached by the chimeric molecule. That would mean only certain genetic diseases could be cured with this technique.

Or the reason could be more mundane: Seemingly minor steps in an experiment can drastically alter the outcome. For example, synthesizing the chimeric molecule is very tricky. Steer used one made by a company in California whereas Strauss used one made by a lab in Germany; conceivably, that could make all the difference.

But Steer might have succeeded because he figured out how to *deliver* the chimeric molecule into cells. After all, if it doesn't get into cells, it can't possibly carry out its task.

Steer is an expert on the liver, in particular a single molecule that sits on the surface of liver cells, tongue-twistingly called the asialoglycoprotein receptor. Steer spent seven years studying that single receptor, which latches on to lactose. So he encased the chimeric molecule inside a shell including lactose, and, like a Trojan horse, that shell carried the chimeric molecule into the liver cells.

"Steer's advance in delivery is equal to the invention of the molecule," says Kmiec, "at least in terms of human health." It also highlights

one of the challenges facing this therapy: Can the molecule be delivered into other cells, such as lung cells, where the cystic fibrosis mutation does its damage, or brain cells, where the Tay-Sachs defect wreaks its havoc?

The good news is that, in theory, there is a key to every cell. The bad news is that there are some genetic diseases, such as muscular dystrophy, that would require the genetic correction to be made in virtually 100 per cent of target cells—a goal beyond the reach of even this technique. And still other diseases, such as Huntington's chorea and many cancers, are caused by changes in many letters of the genetic code; this technique can only change up to three misspellings, and only if the letters are all in a row.

But even if liver cells were the only ones that could be targeted, several horrible diseases might well be cured. Aside from hemophilia, Steer has also successfully corrected the genetic mutation responsible for Crigler Najjar syndrome. That's a rare disease in which the liver is unable to metabolize a bilirubin, a substance in the blood which is toxic at high levels. Within days after birth, infants with this condition turn yellow. If left untreated, their muscles go rigid and the babies die. The only cure is a liver transplant, which often fails. While waiting for a transplant, babies with the disease must lie under intense fluorescent light for hours each day, which helps break down bilirubin.

Since his paper was published, Steer says, "I've had calls from across the country from people who have a son or daughter suffering from a genetic disease, and they want to know if I can help. It breaks my heart, because right now I can't help them. But someday we might be able to."

THE LIVER ALSO metabolizes cholesterol, so targeted gene repair in that organ could prevent heart disease. There are many genetic variations in human beings, in which just one or two genetic letters can spell dramatic health differences. The three known genes that cause early-onset Alzheimer's, for example, can differ from their healthy counterparts by just one misspelling. A single mutation on a gene called K-*ras* can dramatically tilt the odds that a person will get pancreatic cancer. Eventually, this gene-repair method could give everyone the best of all possible genetic constitutions.

And therein lies the scary part. "Can you engineer people?" asks Kmiec. "When you are talking about altering DNA, that is always possible." He laughs and says, "It might be good news if people find it doesn't work on every gene right away."

Blaese, a veteran of brave-new-world questions, is more sanguine. "People have been trying to enhance themselves forever," he says. "Would I run down to the health food store if they proved that extract of rhubarb makes you smarter? I probably would, just like you probably would. So whether one talks about that, or taking growth hormone to get taller, or doing gene therapy, it's the same ethical issue." He pauses. "My view at this stage is pretty simple: I know what disease is, and I try to treat it." V

Research assistance: Sam Bruchey